

## A New Record of *Kaloula* (Amphibia: Anura: Microhylidae) in Shanghai, China

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**Abstract** We discovered a medium-sized species of narrow-mouthed frog of the genus *Kaloula* in June and September 2014 during municipal surveys of amphibians in Shanghai. Three narrow-mouthed frogs were collected in city drains of the Binjiang Forest Park, Shanghai. Based on subsequent examination and morphological characters, these specimens were identified as *Kaloula borealis* (Barbour, 1908). The species was mainly distributed in Northern China and Korea. It is a new amphibian record for Shanghai. We found the visible differences in male secondary sexual characteristics between our specimens and Beijing specimens. *K. borealis* was first described inhabiting urban greenlands which could benefit amphibian conservation in urban and urbanizing areas.

**Keywords** *Kaloula borealis*, Urban greenland, Amphibian, new record

The genus *Kaloula* Gray, 1831 (Anura: Microhylidae) is currently composed of 17 recognized species and is distributed throughout Northern China, Korea, Lesser Sunda Islands, Philippines, Bangladesh, India, and Sri Lanka (Diesmos *et al.*, 2002; Das *et al.*, 2004; Fei *et al.*, 2009; Frost, 2014). This genus is composed of only five species in China (Mo *et al.*, 2013). However, no species of this genus has been reported in the area of Shanghai Municipality. A 3 year wildlife survey in Shanghai Municipality was initiated in June 2013. We found 8 narrow-mouthed frogs (*Kaloula borealis*) after a rainstorm in city drains of Binjiang Forest Park on 22 June 2014 (Site A: 31°23'1" N, 121°31'20" E) (Figure 1). We later counted 51 individuals around a pond of the same park on 4 September 2014, and another 23 individuals in greenlands near a small stream of Wusong Coastal Forest Park next day (Site B: 31°23'38" N, 121°30'23" E). This

species was first described as inhabiting urban greenlands; two females and one male were collected, euthanized, and fixed in formalin, then transferred to 70% ethanol. Muscle or liver tissues were sampled and preserved in 100% ethanol for DNA extraction. All specimens (Voucher Nos.: ECNU14062203; ECNU14062204; ECNU14062205) were archived at the School of Life Science of East China Normal University, China.

Body measurements: SVL (snout–vent length: from the tip of the snout to the vent); HL (head length: distance between jaw angle and snout tip); HW (head width: across jaw angle); SL (snout length: from anterior eye front to snout tip); IND (internarial distance); IOD (interorbital distance); UEW (maximum upper eyelid width); ED (eye diameter); LAHL (length from the elbow to the tip of the third finger); HLL (hind leg length: from the vent to the tip of the longest toe); TL (tibia length); TW (tibia width); FOL (foot length: from the base of the inner metatarsal tubercle to the tip of the fourth toe). We obtained comparative morphological data from previous studies on *Kaloula* specimens and from photographs of live specimens (Table 1). We used Independent *T*-tests to compare our measuring data with previous data. Our results indicate that these morphological parameters of our specimens are consisted with *K. borealis*

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**Table 1** Morphological comparison of *Kaloula borealis* collectiong from Beijing and Shanghai (in mm).

Specimens	ECNU14062203 (♀, <i>n</i> = 1)	ECNU14062204 (♀, <i>n</i> = 1)	ECNU14062205 (♂, <i>n</i> = 1)	<i>Kaloula borealis</i> * (♀, <i>n</i> = 10) Range; Mean	<i>Kaloula borealis</i> * (♂, <i>n</i> = 10) Range; Mean
Locality	Shanghai	Shanghai	Shanghai	Beijing	Beijing
SVL	42.9	41.7	37.3	40.0–45.0; 42.5	40.5–46.0; 43.2
HL	10.8	11.1	10.4	10.5–12.5; 11.6	10.8–12.0; 11.5
HW	12.9	12.9	10.2	12.0–13.5; 12.8	12.8–15.2; 13.9
SL	4.6	4.9	4.6	4.2–5.0; 4.6	4.0–4.5; 4.4
IND	3.1	3.4	3.2	3.0–4.0; 3.3	3.0–3.6; 3.2
IOD	4.6	4.5	4.4	4.5–5.2; 4.9	4.4–6.0; 5.0
UEW	3.1	3.1	2.7	2.8–3.7; 3.2	2.7–3.8; 3.2
ED	4.4	5.1	4.4	4.5–5.9; 5.1	4.5–5.5; 5.1
LAHL	19.6	17.6	17.5	17.0–19.7; 18.8	18.0–22.0; 20.6
HLL	46.1	44.4	40.1	39.5–44.5; 42.2	41.6–49.8; 45.6
TL	13.2	11.4	12.7	11.5–14.0; 12.9	11.5–14.0; 12.9
TW	5.7	5.5	5.3	5.0–5.8; 5.3	4.5–6.3; 5.4
FOL	15.6	14.7	15	13.5–15.8; 14.5	14.0–16.7; 15.2
FOL	15.6	14.7	15.0	13.5–15.8; 14.5	14.0–16.7; 15.2

Note: \*Fei *et al.* (2009).

**Figure 1** (a) Breeding habitat and (b) Reproductive activity of *Kaloula borealis*.

(ECNU14062203: Levene's test  $F = 0.038$ ,  $P = 0.847$ ,  $T$ -test  $P = 0.946$ ; ECNU14062204: Levene's test  $F = 0.001$ ,  $P = 0.972$ ,  $T$ -test  $P = 0.984$ ; ECNU14062205: Levene's test  $F = 0.128$ ,  $P = 0.724$ ,  $T$ -test  $P = 0.757$ ).

We assigned this specimen to *K. borealis* according to the following characteristics: medium size (41.7–42.9 mm in two adult females, 37.3 mm in one adult male), smooth or slightly rough olive dorsum with irregular dark-green marks and brown spots (Figure 2a), half-webbed toes (except for the fourth toe), tips of both fingers and toes

not dilated, obtuse upper surface of the tips of both fingers and toes (Figure 2d), and sides with small lemon-colored spots (Figure 2b). However, these specimens differed from those described by Fei *et al.* (2009) in secondary sexual characteristics. The specimens from Shanghai had flesh-colored throats, no epidermal adhesive glands in the chest and belly, thin and semitransparent ventral skin, and white linea masculina (Figure 2c).

*Kaloula borealis* was found in Binjiang Forest Park and Wusong Coastal Forest Park, which are adjacent to



**Figure 2** (a) Dorsal, (b) dorsolateral, (c) ventral, (d) foot view of adult *Kaloula borealis* (ECNU14062205 (♂)).

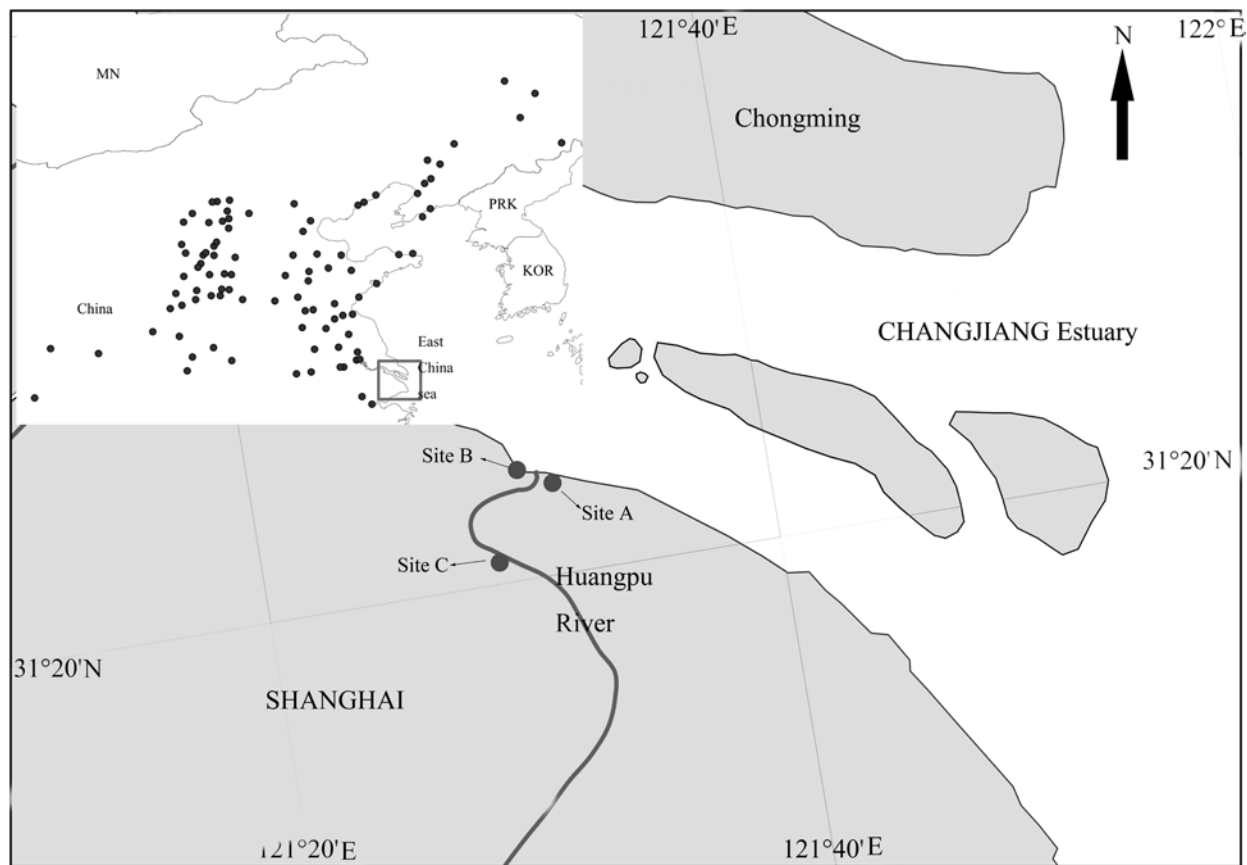
the Changjiang River and to the banks of the Huangpu River, respectively (Figure 3). Local climate is subtropical monsoon, with an annual mean temperature of 16.9°C and average annual rainfall of 1213 mm. These two parks were reclaimed from the Changjiang River in the 1950s and the 1960s. The landscape is mainly secondary forest, with fresh-water ponds connected by a relatively dense short river network. The vegetation is composed of an artificially planted forest predominated by trees, such as camphor tree (*Cinnamomum camphora*), bald cypress (*Taxodium distichum*), pond cypress (*T. ascendens*) and southern magnolia (*Magnolia grandiflora*), the aquatic plants around the wetlands were common reed (*Phragmites australis*), wall iris (*Iris* sp.), great bulrush (*Schoenoplectus* sp.), and shrubs (*Fatsia japonica*, *Ligustrum quihoui*, *Pittosporum tobira*, *Viburnum odoratissimum*). Elevation of sampling sites ranged from 5 to 15 m, with a temperature of 25°C and humidity of 95% as we measured in the field. The habitats of the

frog adjoined the wetlands of the park (Figure 4), and *K. borealis* gathered in the drains for breeding after rainstorm.

## Discussion

We discovered a medium-sized species of narrow-mouthed frog (*K. borealis*) of the genus *Kaloula* in June and September 2014 during municipal surveys of amphibians in Shanghai. This is a new record of the genus *Kaloula* and *K. borealis* in Shanghai. The morphological data and characteristics of *K. borealis* from Shanghai were consistent with Beijing specimens except secondary sexual characteristics. Shanghai specimens have flesh-colored throats, no epidermal adhesive glands in the chest and belly, thin and semitransparent ventral skin, and white linea masculina (Figure 2c). By contrast, Fei *et al.* (2009) described the specimens of Beijing with dark gray throats, epidermal adhesive glands in the chest but not





**Figure 3** Map of Shanghai, big points showing localities of *Kaloula borealis* found in Shanghai. Site A: Binjiang Forest Park; Site B: Wusong Coastal Forest Park; Site C: Fudan University campus in Jiangwan Town. Inset, distribution area of *K. borealis* in China, small points showing localities of *K. borealis* according to published materials.

in the belly, and mulberry-colored masculina. Due to the limited sampling size, we cannot conclude whether the differences between the two populations are significant or not. Molecular phylogeny research will be performed in near future, and identify the difference of secondary sexual characteristics (Matsui *et al.*, 2011).

Our field surveys indicated that *K. borealis* has inhabited the urban greenlands adjoining the mouth of the Huangpu River. In addition, we observed reproductive activity and eggs of *K. borealis* in a pond in Binjiang Forest Park, and this species has formed a population in artificial ponds and stormwater wetlands of urban greenlands. Upon communicating with local ecologists, we learned that one college student found individuals of this species in Fudan University's campus in Jiangwan Town (Site C: 31°20'24"N, 121°30'16"E) in 2008, which is 5 km from Site A. This account was further confirmed by the website of China with *K. borealis*'s picture (Internet reference, 2012). Considering the absence of *K. borealis* during a herpetological survey of Shanghai in the 1990s (Shanghai Forestry Bureau, 2000), we

speculated that this species has only migrated to the urban greenlands of Shanghai in recent years. Previous studies on the herpetology of Shanghai from the 1960s to present indicated that our present work is the first record on *K. borealis* in Shanghai, despite this species being distributed in adjacent provinces, such as Zhejiang and Jiangsu Province (Fei *et al.*, 2009). We also discovered evidence of this frog using artificial greenlands for breeding along the Huangpu River in Shanghai. The number of known amphibian species was up to 13 in Shanghai, including *Bufo gargarizans*, *B. raddei*, *Hyla immaculata*, *H. chinensis*, *Hylarana guentheri*, *Fejervarya multistriata*, *Pelophylax nigromaculatus*, *Pelophylax plancyi*, *R. japonica*, *Hoplobatrachus chinensis*, *Microhyla ornata*, *Rhacophorus dennysi*, and *Polypedates megacephalus*, according to the records of 1980 (Huang *et al.*, 1980). However, only 8 species of frogs had been found in the first wildlife survey of Shanghai Municipality in 2000 (Shanghai Forestry Bureau, 2000), with *B. raddei*, *H. chinensis*, *H. guentheri*, *R. dennysi*, and *P. megacephalus* still missing during surveys. From 2013 to 2014, the



**Figure 4** Habitat of *Kaloula borealis*.

second wildlife survey of Shanghai Municipality was undertaken. During these surveys, we counted 5 species frogs (*B. gargarizans*, *F. multistriata*, *P. nigromaculatus*, *P. plancyi* *M. ornata*) and 1 new record species (*K. borealis*). By contrast with the surveys in 2000, we did not encounter *H. immaculata*, *H. chinensis* and *R. japonica* in these surveys.

Urbanization is recognized as a major driver of amphibian declines globally (Hamer *et al.*, 2008). Urban greenlands is useful for mitigating habitat loss and retaining water runoff from impermeable urban surfaces, and serve as breeding habitat for amphibians. Our studies show that urban greenlands play increasingly important roles as an amphibian refuge due to urbanization in the Changjiang Estuary. Amphibians require aquatic habitats to support larval development and terrestrial habitat for adult stages. Urban greenlands provide necessary habitat for many species that spend breeding season in ponds, but forage and hibernate in the surrounding riparian zone and upland vegetation (Scheffers and Paszkowski, 2013). In areas with high levels of human activity, urban gardens and greenland parks are likely the last available habitats for amphibians and have tremendous value in preserving the diversity of amphibian species. Moreover, *K. borealis* is distributed along the two banks of the Huangpu River. We hypothesize that the river plays a key role in dispersal, and most researchers agree that rivers help create and sustain species diversity (Lougheed *et al.*, 1999; Li *et al.*, 2009). With further study and extensive field work, we speculate that *K. borealis* may be found in more localities, which will be useful to understand the exact distributional pattern of *K. borealis* in Shanghai.

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